Saskatchewan Agriculture, Food and Rural Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

19990105

BIOCONTROL OF HEAD AND ROOT DISEASES CAUSED BY FUSARIUM SPP. USING PLANT GROWTH PROMOTING RHIZOBACTERIA IN WHEAT AND BARLEY

Funded by:
The Agriculture Development Fund

March 2003
Prepared by: University of Saskatchewan

104-00705/

Date: February 2003
Revised: September 2003
ADF # 19990105
(M.R. Fernandez)

Final Report

Biocontrol of head and root diseases by Fusarium spp. using plant growth promoting rhizobacteria in wheat and barley

Prepared by
K. G. Hanson, M.Sc.
Semiarid Prairie Agricultural Research Centre (SPARC),
Agriculture & Agri-Food Canada, AAFC
P.O. Box 1030, Swift Current, SK
S9H 3X2

1. Executive Summary

K. G. Hanson began work on this project as of April 2, 2001 taking over from Dr. M. Banerjee. Some records and bacterial cultures were obtained from Dr. Banerjee prior to his departure. At that time, a shift in the focus of the study was implemented to increase the chances of success. Originally mostly rhizobacteria were isolated for use as potential biological control agents (BCA) to reduce Fusarium diseases in cereals specifically targeting Fusarium head blight (FHB) pathogens. After April 2001, bacterial isolates from cereal crop residues and mature plant material were used to investigate reductions in FHB pathogens in cereal crop residues.

This study found 58 bacterial isolates that have *in vitro* antagonistic activity against plant pathogenic fungi commonly associated with wheat and barley. Of these isolates twenty were found to have shown control of more than one of the test fungal species. Further trials showed that sixteen of the bacterial isolates controlled vegetative growth of a number of *Fusarium graminearum* isolates from across the Canadian prairies. Fungal isolates were found to have very poor antagonistic control of most pathogenic fungi *in vitro* and were not pursued as potential biological control agents after initial screening. The top twenty bacterial isolates will be stored at ultra-low temperatures to ensure viability and efficacy is maintained for the culture collection. The ability of the bacterial cultures to grow after storage further reduced the number of bacterial isolates tested in later trials until four isolates were selected as the best candidates for further work.

A spring wheat nursery was established and harvested for use in later laboratory and growth chamber studies. A portion of the nursery was inoculated with *F. graminearum* for use in efficacy studies. Uninoculated crop residues from this nursery have been used to evaluate bacterial population survival for some of the bacterial cell-lines. The bacterial isolates tested in the initial survival trial survived at relatively high levels for at least one month on crop residues. Background saprophytic fungal levels made evaluation of the treatments impossible on these residues and the material was sterilized for use in further assays.

The selected bacterial isolates were used in efficacy trials to evaluate their ability to reduce populations of *F. graminearum*. An initial efficacy trial was conducted using *F. graminearum* inoculated straw from the wheat nursery however the high number of non-*Fusarium* saprophytic fungi masked the population of the target fungal pathogen, as described above. All the bacterial treatments were shown to reduce overall fungal numbers compared to a control after 14 days of incubation. A second efficacy assay was conducted using wheat straw that had been sterilized and then subsequently inoculated with *F. graminearum* or *F. avenaceum* strains that had been used in the previous screening assay. This second assay allowed both efficacy and survival of the bacterial isolates to be evaluated from the same treatments by counting fungal colonies on potato dextrose agar and bacterial colonies on tryptic soy agar. From this assay, isolate B60 emerged as the best bacterial treatment with good survival and reduction in

F. graminearum or *F. avenaceum* populations on crop residues. Under high moisture content conditions in a final assay with *F. graminearum*-infested straw, the selected bacterial isolates all suppressed the population of fungi recovered compared to the control.

Isolate B60 performed well under all conditions tested, controlling all fungal species it was tested against *in vitro* and on wheat straw, and always persisting at high population on wheat straw. This isolate should be considered as a good candidate for biological control of *Fusarium* spp. associated with diseases of cereal crops.

2. Technical Report

2.1 Project Objectives

To screen, bioassay and identify soil-borne bacteria from soil and crop residues as biocontrol agents for Fusarium head blight and other diseases in wheat and barley.

After Dr. Banerjee's departure at the end of March 2001, K. G. Hanson took over this project after consultations with Dr. M. R. Fernandez. Records and cultures recovered from Dr. Banerjee's previous work have been utilized for some of the ongoing research. The project was expanded to include other sources of potential biocontrol agents including bacteria from soil, crop residues, mature plant material and the existing rhizobacterial culture collection. Also, more plant pathogenic fungi were used during initial screening or re-screening. The reason for the additions is that these fungal pathogens cause various diseases in cereals which are important to control and if a wide spectrum biological control agent is identified it may ultimately be of greater and more widespread use.

2.2 Project Activities

2.2.1 Materials and Methods

Plant Pathogenic Fungi:

Two isolates of *Fusarium graminearum* were used in the initial screening process. In addition to the *F. graminearum* isolates, ten other fungal isolates were used, two each of the following five species:

F. avenaceum,
F. culmorum,
F. pseudograminearum,
Pyrenophora tritici-repentis, and
Cochliobolus sativus.

These fungal isolates were obtained from the plant pathology laboratory of the Semiarid Prairie Agricultural Research Centre (SPARC). The fungi had been obtained from producers' fields across Saskatchewan in the 1998, 1999 and 2000 growing seasons. All fungal isolates were maintained on potato dextrose agar (PDA) medium at 5°C. The active fungi's hyphal growth was used as inoculum for the *in vitro* screening of the bacterial isolates.

In the fall of 2001, twenty more isolates of *F. graminearum* were obtained from Mr. Randy Clear of the Canadian Grain Commission's Mycology Laboratory. Ten of these *F. graminearum* isolates were from Manitoba while the remaining ten were from Alberta. These twenty isolates were then used for further *in vitro* screening of the potential biological control agents.

Recovery of Bacterial Isolates from Culture Collection:

Over 500 bacterial isolates, both from new isolations and an existing culture collection at SPARC, were initially tested for antagonism of *F. graminearum*. The vast majority of these were rhizobacteria and were found to have no antagonistic activity on the test fungi. Many of these bacterial cultures had been maintained on agar slants at 5°C. Those bacteria identified from the laboratory records obtained from Dr. Banerjee as isolated from crop residues, the phyllosphere or heads of wheat or barley were initially tested for viability. Of the 79 isolates tested for growth on trypticase soy agar (TSA), 44 were found to be viable. These bacterial isolates were then subcultured to ensure purity of cell line. Several rhizobacterial isolates had also shown some antagonism to fungal growth *in vitro* and these were located in the culture collection to test for viability. The fifteen rhizobacterial isolates that showed fungal antagonistic activity and were still viable were re-streaked to check for purity.

Isolation of New Bacterial and Fungal Isolates for *in vitro* Screening:

Barley and spring wheat crop residues collected during field surveys conducted in August of 2000 by Dr. M.R. Fernandez were sampled. Approximately 3 g of the samples were then placed in deep petri dishes with 2 ml of sterile, reverse-osmosis (R-O) water. The petri dish was then sealed with Parafilm to prevent desiccation and incubated at 21°C in the dark for seven to ten days. Incubated crop residues were subsampled (approximately 1 g) which was then used in a dilution series to the 10^{-4} dilution. To obtain fungal isolates the 10^{-1} and 10^{-2} dilutions were plated onto rose bengal streptomycin agar (RBSA). To obtain bacterial isolates the 10^{-3} and 10^{-4} dilutions were plated onto tryptic soy agar (TSA). Both the RBSA and TSA plates were incubated inverted in the dark at 21°C. After three days the RBSA plates were evaluated for growth and individual fungal colonies were chosen for subculture onto PDA. The TSA plates were examined after ten to fourteen days and individual bacterial colonies streaked onto TSA to ensure cell-line purity.

Initial Screening of Bacterial and Fungal Isolates:

Bacterial isolates recovered from the culture collection and from new isolations were then used in the *in vitro* screening assay. Up to four bacterial isolates were inoculated near the periphery of PDA plates that were then incubated, inverted in the dark at 26°C, for three to four days prior to the addition of the fungi. Two replications of each bacterial isolate combination were used for each of the twelve fungal isolates. After addition of the fungal pathogen isolates, by adding a 3 mm plug of actively growing mycelium, the plates were re-incubated as indicated above. The screening plates were observed after 3 days and thereafter as needed to observe and record fungal antagonism by the bacterial cultures (Plate 1). The growth rates of the different fungal pathogens varied considerably

with most *Fusarium* spp. growing to the plate edge within five days or less while the *P. tritici-repentis* took a week and *C. sativus* over two weeks.

Those bacterial isolates that exhibited any fungal antagonism were recorded and kept for further studies. The zone of inhibition, i.e. the area around the bacterial colony where no fungal hyphae grew, varied considerably between isolates, however the size of this zone may not indicate the ability of the bacteria to control the fungi outside of the *in vitro* screening assay. Those bacteria with very small zones of inhibition or not overgrown by the plant pathogenic fungi may have been producing water-insoluble antifungal metabolites and were kept for further work (S. Boyetchko, personal communication).

The newly isolated fungi for evaluation as potential BCA were screened for *in vitro* antagonism against the fungal pathogens on PDA. A 3 mm plug of test fungal isolates were placed at the periphery of a PDA plate, with only one test fungi per plate, and were then incubated, inverted in the dark at 26°C. After two days, the pathogenic fungal isolates were placed on the plates across from the test fungal isolates. The plates were then re-incubated under the same conditions for three days before evaluating *in vitro* antagonism and then reevaluated thereafter as required. Fifty fungi were originally isolated for screening however only 40 grew after subculture. Only twelve fungal isolates showed any pathogen antagonism and of these only two inhibited *F. graminearum*. The antagonism exhibited by these fungi was very weak and due to the poor growth upon subculture, all fungal isolates were dropped from any further *in vitro* assays.

Culture Collection Storage:

Maintenance of bacterial isolate activity is paramount in the success of this project. To meet this objective, the culture collection will be kept at ultra-low temperatures in cryostorage (-80°C). Cryostorage has been shown to achieve long-term storage for many bacterial cultures without loss of viability or activity. The top twenty bacterial isolates were placed in cryostorage.

Screening of select bacterial cell-lines against several *F. graminearum* isolates:

Seventeen of the twenty bacterial isolates were recovered from cryostorage and used for inoculation of 100 ml of TSB in flasks. The other three isolates in cryostorage were not recovered for testing due to relatively poor antagonism against *F. graminearum* in the initial screening process. The inoculated broth flasks were incubated at ambient room temperature (22°C) in the dark on a platform orbital shaker oscillating at 200 rpm for at least 48 h. Inoculum from the resulting cultures were used for *in vitro* trials against the *F. graminearum* isolates from Manitoba and Alberta that were described earlier. For this assay only two

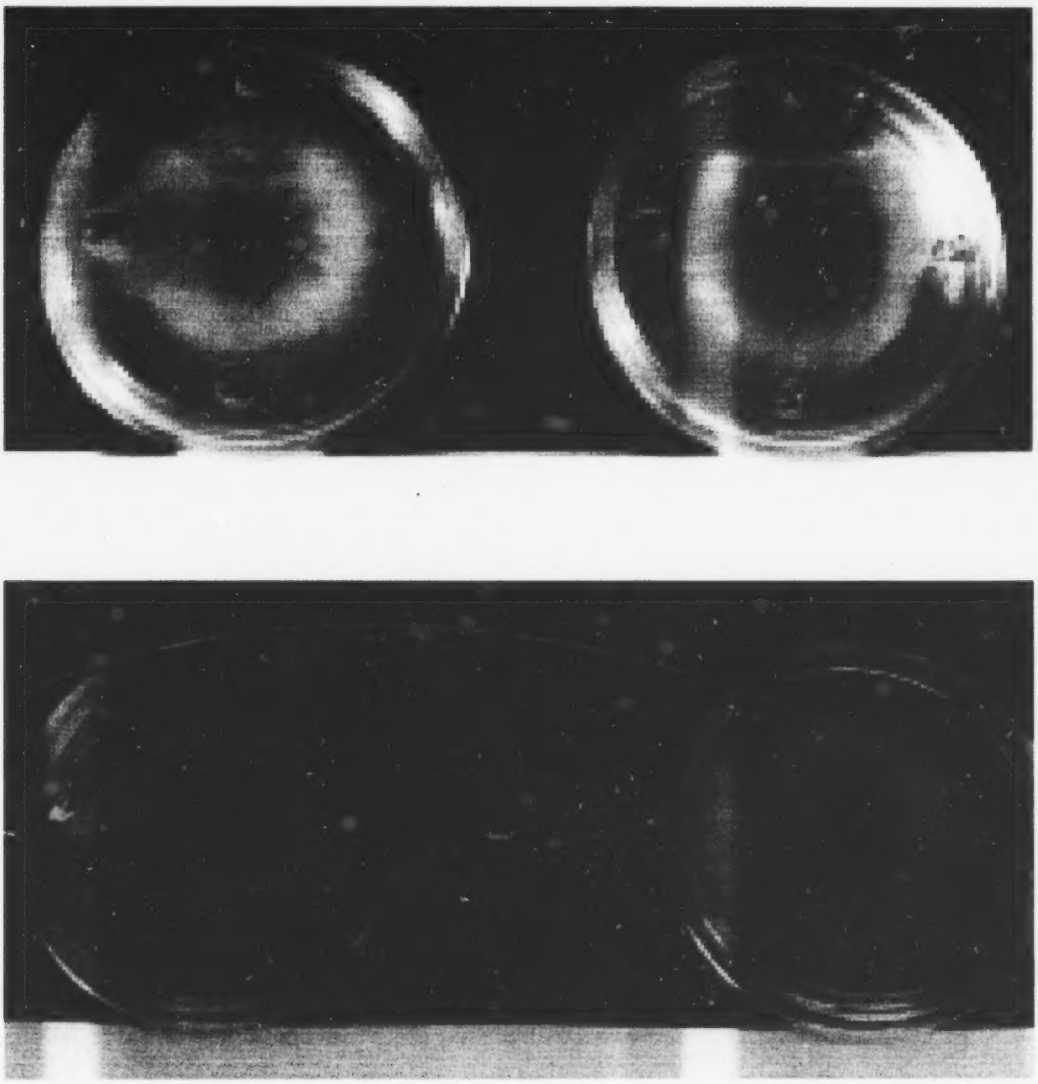

Plate 1. Photographs showing the *in vitro* screening assay. The bacterial isolates are inoculated around the PDA medium's periphery and the fungal isolate at the plate's center. Bacterial isolate 148 shows good fungal antagonism in the top photo. *Fusarium pseudograminearum* is denoted by Fg I, *F. graminearum* by Fg II and *F. culmorum* by Fc.

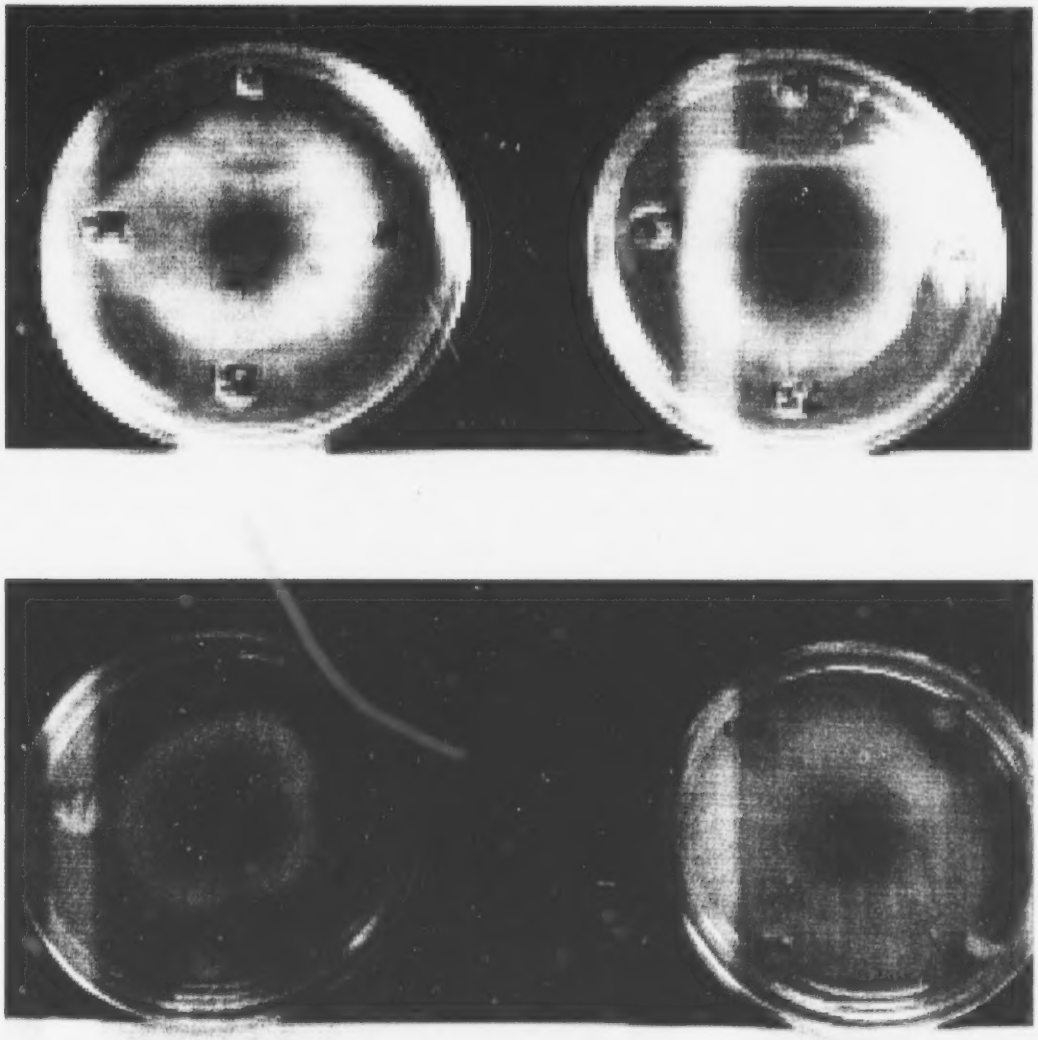

Plate 1. Photographs showing the *in vitro* screening assay. The bacterial isolates are inoculated around the PDA medium's periphery and the fungal isolate at the plate's center. Bacterial isolate 148 shows good fungal antagonism in the top photo. *Fusarium pseudograminearum* is denoted by Fg I, *F. graminearum* by Fg II and *F. culmorum* by Fc.

bacterial isolates were placed on the plate periphery except isolates 260, 564 and B48 which had previously shown spreading growth in the initial screening tests. Isolate B48 failed to grow on the PDA plates after recovery from cryostorage and was therefore excluded from the trial. The bacterial cultures were inoculated onto PDA three days before the addition of the fungal inoculum that was placed into the center of the plate. Incubation and evaluation was conducted following the same procedure as previously described.

Wheat Nursery:

In late May 2001, a spring wheat nursery was established in a growth chamber at SPARC. Spring wheat, cv. AC Barrie, was seeded to 500 milk cartons packed with soil. The wheat was grown until maturity however some of the plants were inoculated with *F. graminearum* at approximately "late dough" stage. The selected plants were manually sprayed with a spore suspension of mixed isolates of *F. graminearum* and the inoculated plants were then incubated in a high humidity misting chamber for 24 h. The plants were then taken to the greenhouse to complete maturation. Once the plants were mature and air-dried, the heads were removed and the crop residue chopped into approximately 2.5 – 7.5 cm lengths, placed into brown paper bags and stored at 5°C. Uninoculated wheat plants were treated in the same manner except for the high humidity incubation. This material was used in laboratory studies and will be used for further studies in the laboratory and growth chamber.

Bacterial Survival on Crop Residues:

The survival of the potential biological control agents in the environment is essential to their ability to be effective therefore persistence on crop residues may be important. An assay to evaluate the survival of select bacterial cultures was initiated on crop residues. Thus far, four bacterial cultures, isolates 177, 181, 260 and 564, have been used in this assay. The bacterial cultures were recovered from cryostorage and used for inoculation of TSB in flasks. The inoculated flasks of broth were incubated for 48 h at 22°C in the dark on an orbital shaker set at 200 rpm. The resulting broth cultures were then used to inoculate the crop residues. Crop residues (straw) from the AC Barrie wheat nursery were sterilized in an autoclave prior to inoculation with the bacterial cultures. Sterile crop residues were placed into sterile deep petri dishes and then weighed to determine the amount of residue per plate. For each g of crop residue one ml of broth culture inoculum was added. Once the inoculum was applied the petri dish was sealed and manually shaken to distribute the inoculum on the residues as evenly as possible. An uninoculated check treatment was prepared using sterile broth resulting in a total of five treatments. Three replicates were used in this assay. The treated crop residues were incubated at 21°C in the dark and to evaluate bacterial survival the crop residues were subsampled periodically, with the weight of subsample determined. The subsamples were then macerated in a blender for two minutes with peptone-phosphate diluent. The

resulting dilution was then placed into a dilution series for plating of the residues to enumerate the number of colony-forming units (CFU) that could be recovered. Selected dilutions were spreadplated onto TSA plates that were in turn incubated inverted at 21°C in the dark. After three days of incubation, the plates were evaluated and the number of CFU counted. Colony morphology was also evaluated to ensure that no other bacteria contaminated the crop residue samples. The resulting CFU counts were used to calculate the CFU recovered per g of straw. The crop residues were sampled and plated at time 0 (immediately after inoculation), after one day, three days, seven days, two weeks and three weeks.

Bacterial Efficacy on Crop Residues:

The ability of the potential biological control agents to reduce the populations of the target pathogenic fungi in the environment is the goal of this research. Assays were conducted to evaluate the ability of select bacterial isolates to control *F. graminearum* on wheat crop residues (straw) under controlled conditions. From results of the previous assays, bacterial isolates 148, 260, B60 and B71 were chosen for these evaluations. The bacteria were grown in TSB as described previously with the exception that isolate 148 was inoculated into broth 24 h earlier due to slower growth.

In the initial efficacy trial, straw from the AC Barrie wheat nursery that had been inoculated with *F. graminearum* was placed into deep petri dishes, inoculated with the bacterial broth cultures and incubated using the same protocol as described for the survival assay. The treatments were evaluated by serially diluting subsamples taken at Time 0 and after 3, 7 and 14 days of incubation. The appropriate dilutions were plated onto mPDA, incubated for 3 to 5 days and then fungal CFU counted.

Dual Assay on Crop Residues:

Upon reaching this stage, it was felt by the investigators that this assay was not adequate for evaluating the objective (evaluating reduction of *F. graminearum* and other target fungi populations) therefore a second approach to the assay was required. This second assay began by autoclaving straw from the nursery and then inoculating this sterile straw with *F. graminearum* isolates previously used in the screening assays. The *F. graminearum* inoculum was added from colonies actively growing on PDA. Approximately 30 g of sterile straw was placed in a foil pan, inoculated and then 30 ml of sterile water added. The inoculated straw in the pan was then incubated in a clear plastic bag at 21 C with 16 h photoperiod for 14 days until visibly heavy fungal growth was observed. The straw was then mixed under aseptic conditions and re-incubated for a further week under the same conditions. This inoculated straw was then used for the second efficacy assay. The same bacterial isolates used in the first efficacy assay were used in the second and were prepared as previously described. The

assay followed the protocol previously described with two exceptions: the treatments were incubated at 21 C with a 16 h photoperiod and, both fungal CFU (for efficacy evaluation) and bacterial CFU (for survival evaluation) were counted after plating with appropriate dilutions on mPDA and TSA, respectively.

2.2.2 Results and Discussion

Initial Screening of Bacterial and Fungal Isolates:

Of the viable bacterial cell-lines from the culture collection tested, 28 isolates were found to have *in vitro* antagonism to the fungal pathogens. Eleven of these isolates inhibited the mycelial growth of three or more of the fungal pathogens and these were placed into cryostorage for further testing. The remaining 17 were maintained on TSA plates at 5°C. Ninety new bacteria isolates taken from crop residues were initially screened. From this screening, 30 were found to exhibit fungal pathogen antagonism. Nine of these new isolates were found to inhibit four or more fungal pathogens and were placed into cryostorage. The remaining 21 isolates were maintained on TSA plates at 5°C. The bacteria placed into cryostorage are listed in Table 1.

Table 1. Select bacterial isolates from initial screening and their fungal antagonism *in vitro*

SOURCE	BACTERIAL ISOLATE	NUMBER OF FUNGI INHIBITED (12 TESTED)
Wheat heads	148	12
Wheat heads	260	12
Barley rhizosphere	564	12
Wheat residue	B22	12
Wheat residue	B48	12
Wheat heads	B60	12
Wheat residue	117	11
Barley residue	B75	9
Wheat residue	B61	7
Wheat residue	B71	7
Barley residue	171	6
Wheat residue	177	6
Wheat residue	B14	6
Wheat residue	B17	6
Wheat residue	B57	5
Barley residue	161	4
Barley heads	181	4
Wheat rhizosphere	625	4
Wheat residue	159	3
Barley residue	160	3

Screening of select bacterial cell-lines against several *F. graminearum* isolates:

Most of the bacterial isolates used in this assay showed good to excellent *in vitro* inhibition of mycelial growth of the *F. graminearum* isolates (Table 2). Nine isolates were antagonistic to all *F. graminearum* isolates tested against while all the remaining seven bacterial cultures inhibited at least 11 of the 20 fungal isolates. Four isolates, B60, B71, 148 and 260, clearly inhibited all the fungal isolates tested against regardless of source and to a greater degree than the other bacterial cultures. The zones of inhibition by the bacteria were clearly observed on many of the agar plates (Plates 2 & 3).

Table 2. *In vitro* antagonism of *Fusarium graminearum* isolates from different source locations by select bacteria

BACTERIAL ISOLATE	NUMBER OF FUNGI INHIBITED		
	Manitoba Sources (10)	Alberta Sources (10)	Total (20)
148	10	10	20
260	10	10	20
B60	10	10	20
B71	10	10	20
161	10	10	20
171	10	10	20
B22	10	10	20
B57	10	10	20
B75	10	10	20
181	10	9	19
B14	9	10	19
B61	8	10	18
177	8	7	15
B17	4	9	13
564	7	5	12
117	5	6	11

Bacterial Survival on Crop Residues:

Inoculation of straw with the bacterial cultures tested resulted in good initial colonization (Figure 1). The ability of bacteria to quickly colonize surfaces is an important characteristic that may improve a biological control agent's chances of success. CFU recovered were increasing for three of the bacteria after one week while the population of the other culture (isolate 181) appears to have peaked. After the first seven days, the number of CFU recovered dropped for all the bacterial cultures and at 28 days most appear to be leveling off in population on

Plate 2. Photographs showing the control of fungal mycelium growth of *Fusarium graminearum* isolates from Manitoba by bacterial cultures 260 (top) and B60. Note the large zone of fungal inhibition by 260.

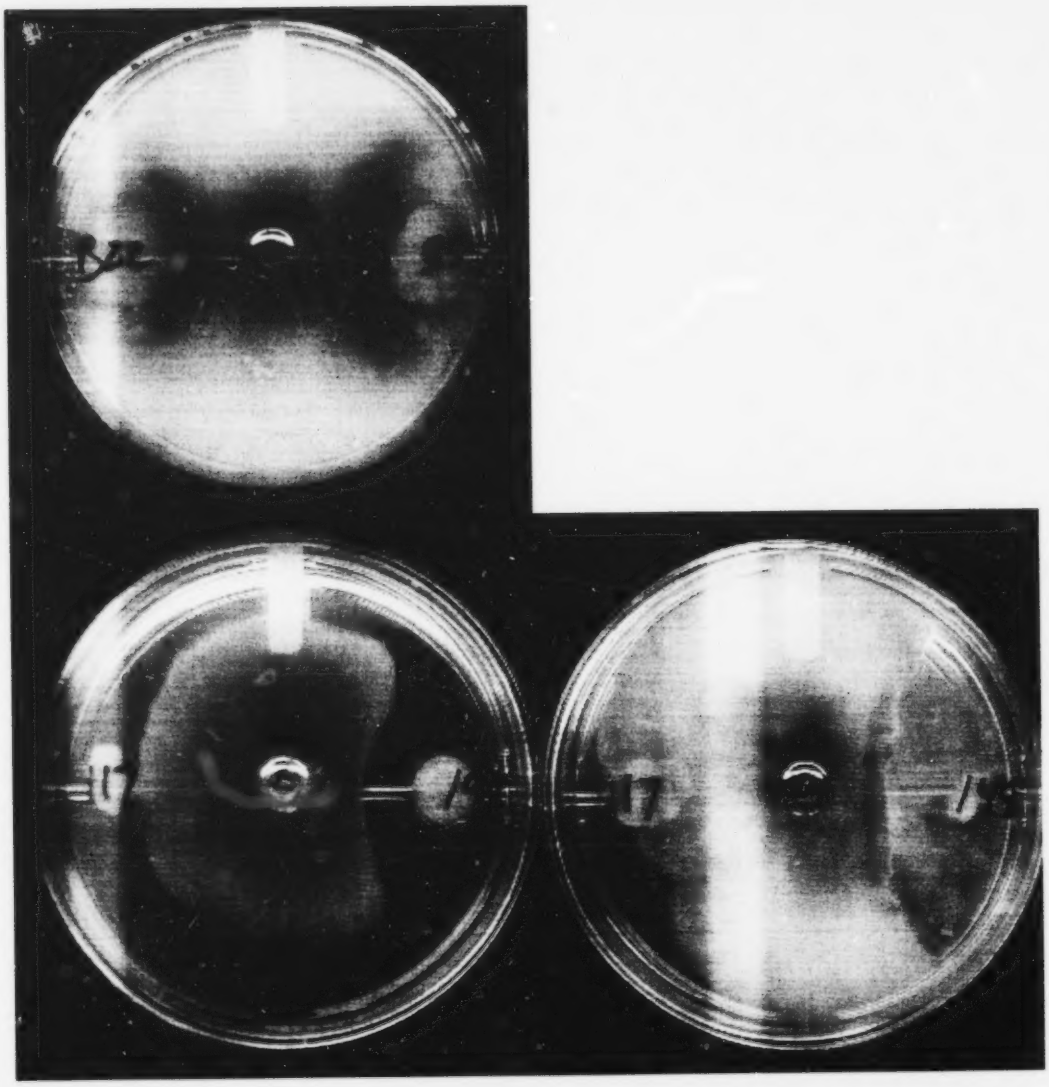

Plate 3. Photographs showing the control of fungal mycelium growth of *Fusarium graminearum* isolates from Alberta by select bacterial cultures. Note the variety of growth patterns exhibited by the fungal isolates when interacting with the different bacterial cultures.

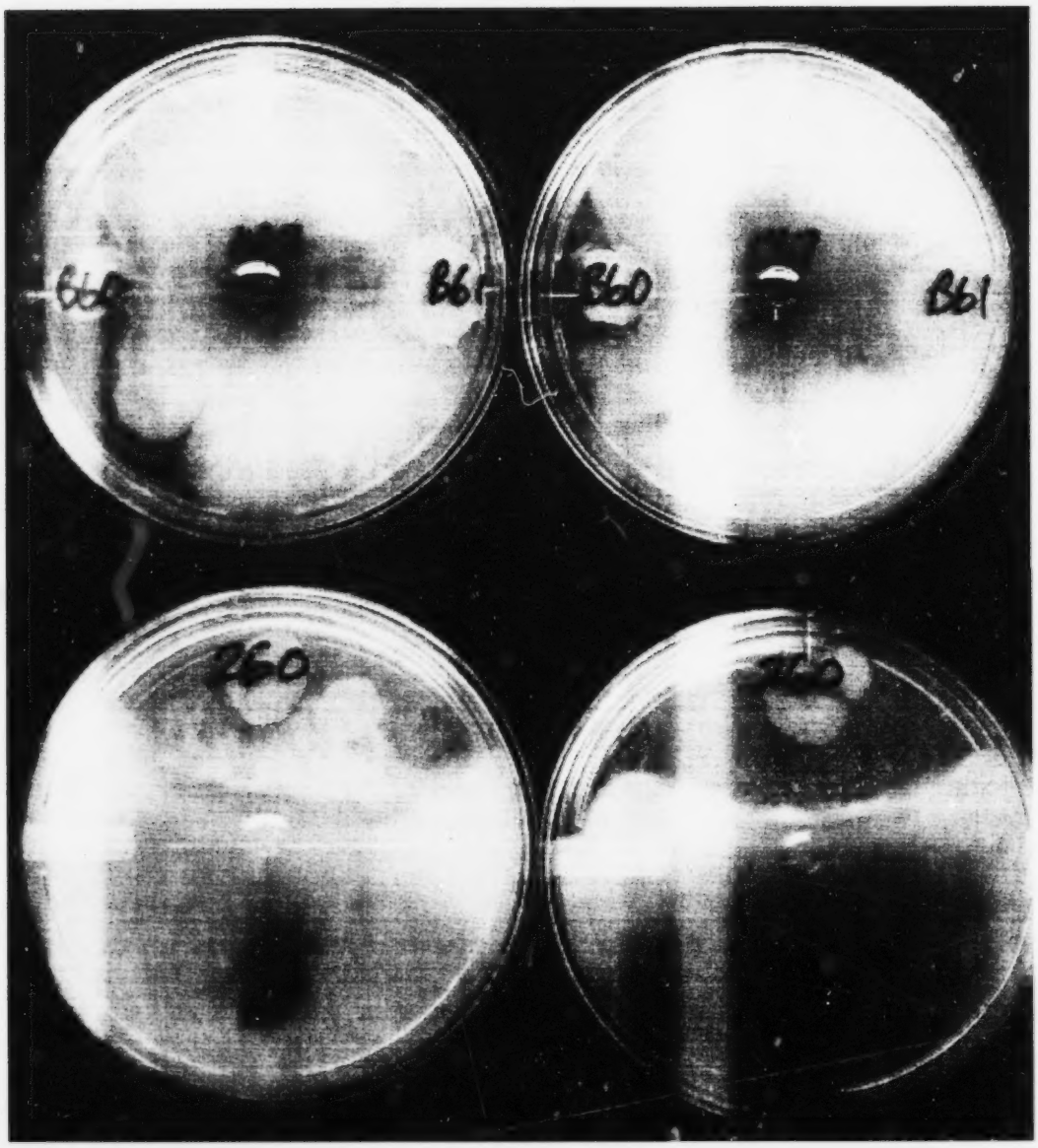

Plate 2. Photographs showing the control of fungal mycelium growth of *Fusarium graminearum* isolates from Manitoba by bacterial cultures 260 (top) and B60. Note the large zone of fungal inhibition by 260.

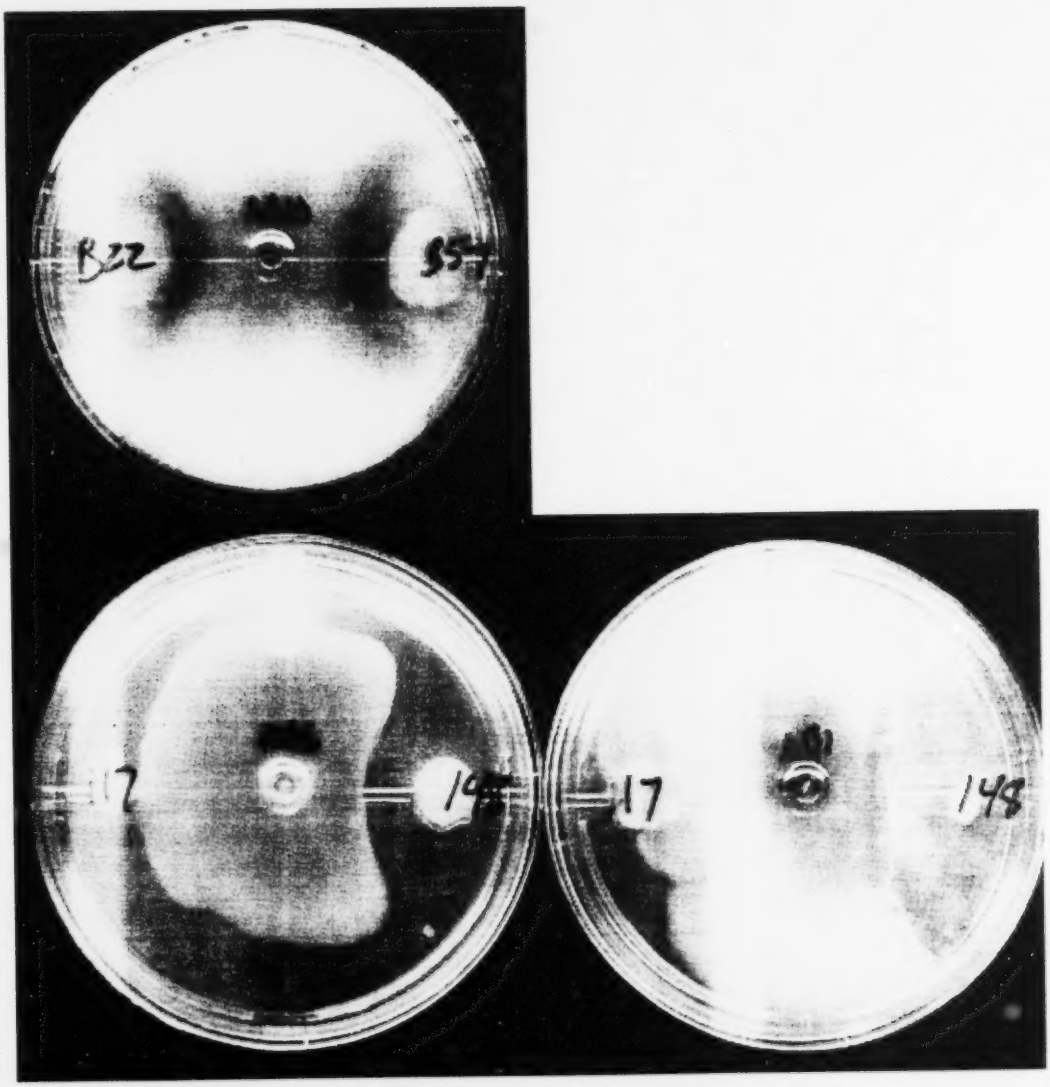

Plate 3. Photographs showing the control of fungal mycelium growth of *Fusarium graminearum* isolates from Alberta by select bacterial cultures. Note the variety of growth patterns exhibited by the fungal isolates when interacting with the different bacterial cultures.

the straw. Isolate 260 is the exception with a large increase in CFU recovered at 28 days compared to the population at 14 days. After this evaluation, it was determined that future work would focus on four top isolates, instead of random groupings of the bacteria, for the remainder of the study due to time constraints.

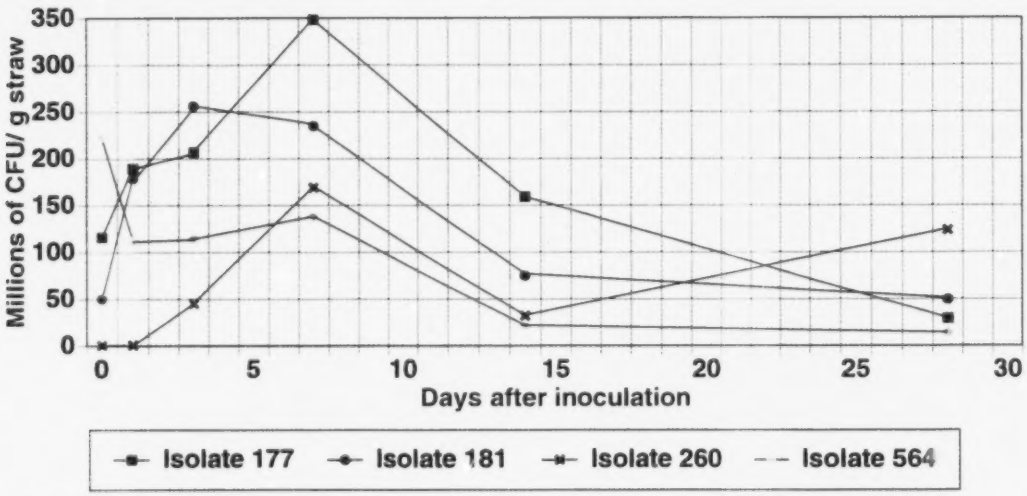

Figure 1. Number of colony-forming units (CFU) recovered from inoculated AC Barrie spring wheat straw over time for selected bacterial isolates being evaluated as potential biological control agents of cereal pathogenic fungi.

Bacterial Efficacy on Crop Residues:

From the initial efficacy trial, using non-sterile straw, it was found that due to the high numbers of saprophytic fungi present on the straw that selective counting of *F. graminearum* was impossible. Thus total fungal CFU were evaluated. The ability of the select bacteria to reduce fungal populations in this assay was variable compared to the control (Figure 2). The available water and nutrients added with the broth cultures might have stimulated fungal growth in some cases. Bacterial isolates 148 and B71 had considerably greater CFU compared to the control after 3 days incubation while isolates 260 and B60 showed lower fungal CFU recovered. Day 7 data was discarded due to medium contamination. By 14 days, all the bacterial treatments had reduced the fungal CFU compared to the control.

Dual Assay on Crop Residues:

This assay was found to work very well to evaluate both the efficacy of the selected bacterial isolates on target plant pathogenic fungi as well as determining their ability to survive on cereal straw.

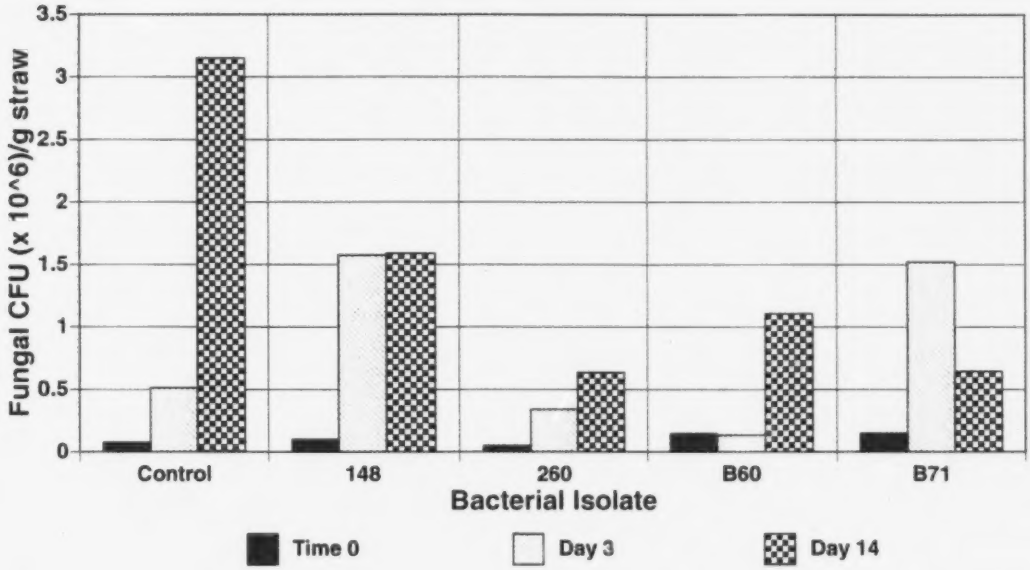

Figure 2. Recovery of total fungal colony-forming units (CFU) from Fusarium graminearum infected AC Barrie spring wheat straw after treatment with select bacterial isolates.

The ability of the select bacterial isolates to reduce the *Fusarium graminearum* CFU recovered from the crop residues varied considerably (Figure 3). After 3 days, 148 showed relatively poor control compared to the other isolates however it increased to approach 80% suppression of the fungus by 14 days before again

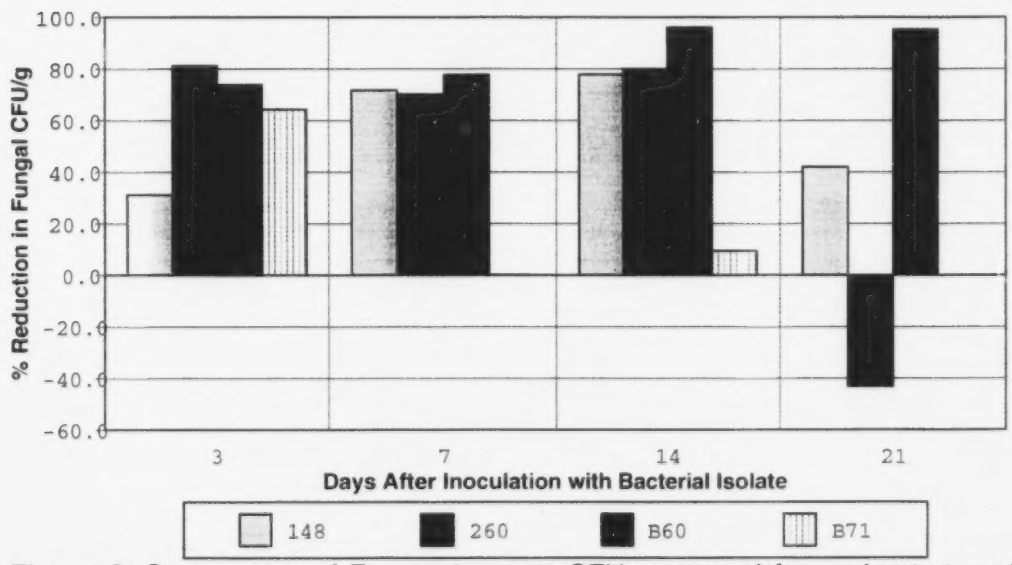

Figure 3. Suppression of F. graminearum CFU recovered from wheat straw by select bacterial isolates.

dropping off. Isolate 260 showed relatively high levels of suppression until 21 days after inoculation when it dropped right off and in fact seemed to enhance the fungal population. B71 rapidly lost efficacy for suppressing the fungal CFU however this may be attributed to a steady decline in its population (Figure 4). Isolate B60 showed a consistently high level of fungal suppression while maintaining a large bacterial population (Figures 3 & 4).

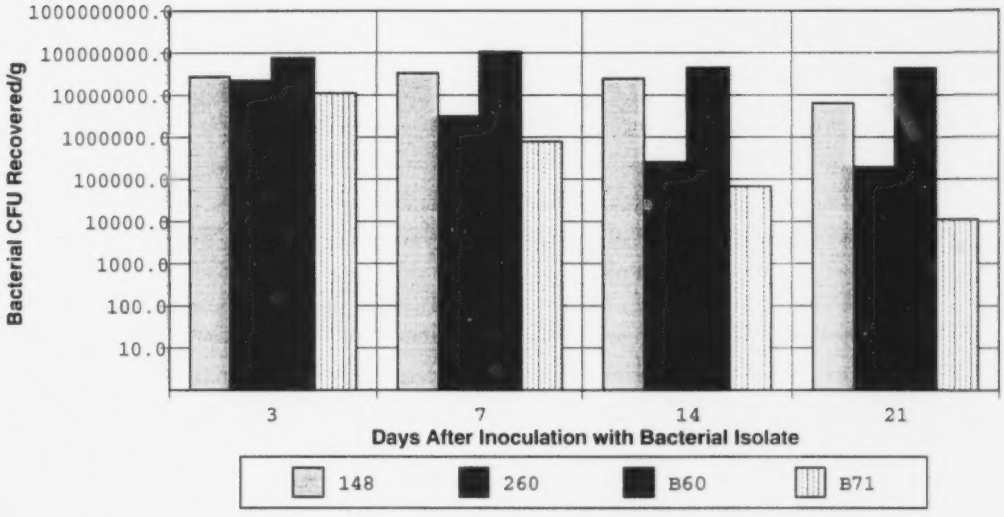

Figure 4. Bacterial populations over time on wheat straw infected with *F. graminearum*.

Survival by the select bacterial isolates was evaluated up to 21 days on *F. graminearum* infected wheat straw. The bacteria were found in relatively high numbers after 3 days of incubation except isolate B71 that was markedly reduced compared to the others, a trend that continued throughout the entire assay. By day 7, isolate 260 was found to be rapidly dying off compared to isolates 148 and B60. After 21 days, isolate B60 was maintaining a relatively consistent population on the *F. graminearum* infected straw while isolate 148 showed significant reductions in population and isolate 260 showed a highly significant reduction in survival.

These isolates were also evaluated on *Fusarium avenaceum* infected straw for efficacy and survival. The ability of the bacteria to suppress the fungus was inconsistent over time except for B60 which retained a consistently high level of fungal control (Figure 5). Once again, B60 had a large population for over a month (Figure 6). The other bacterial isolates had greatly reduced population by day 35.

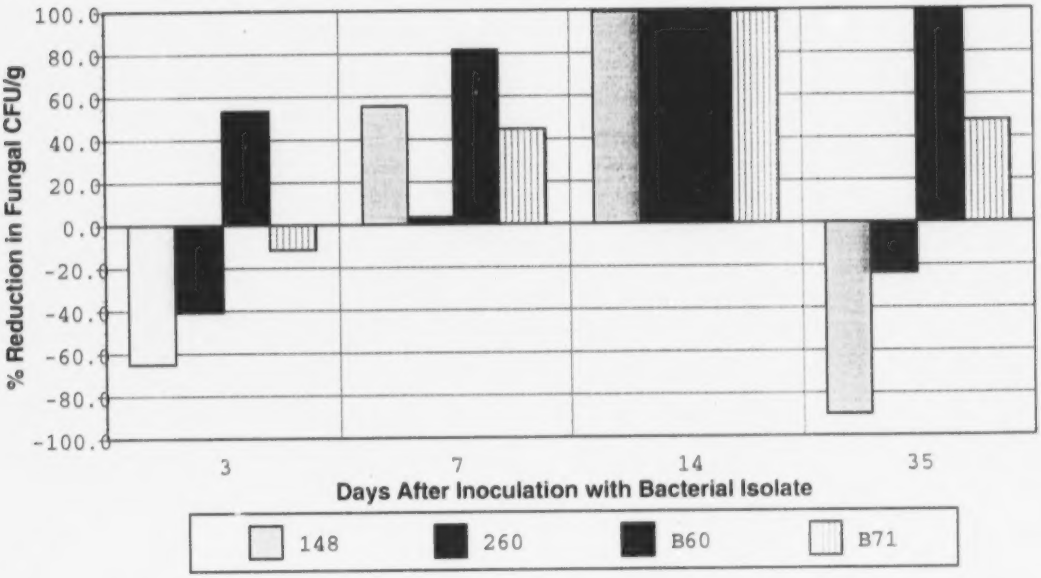

Figure 5. Suppression of F. avenaceum CFU recovered from wheat straw by select bacterial isolates.

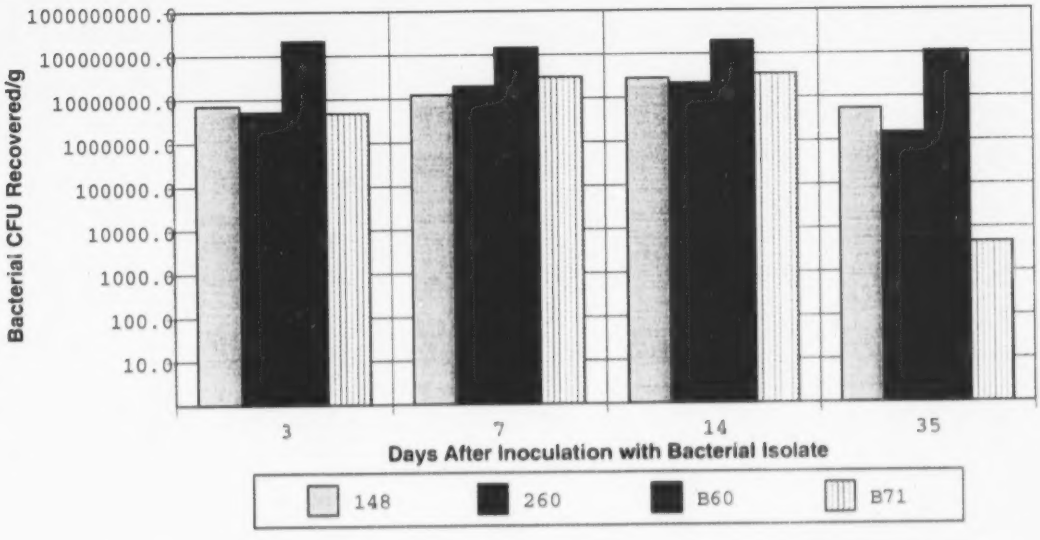

Figure 6. Bacterial populations over time on wheat straw infected with *F. avenaceum*.

A high moisture dual assay was conducted on F. graminearum infected straw. This assay showed a general rise in suppression by the bacteria over a month's incubation (Figure 7). This corresponds with no rapid reductions in bacterial populations that was found with some of the isolates in the earlier, lower moisture assays (Figure 8).

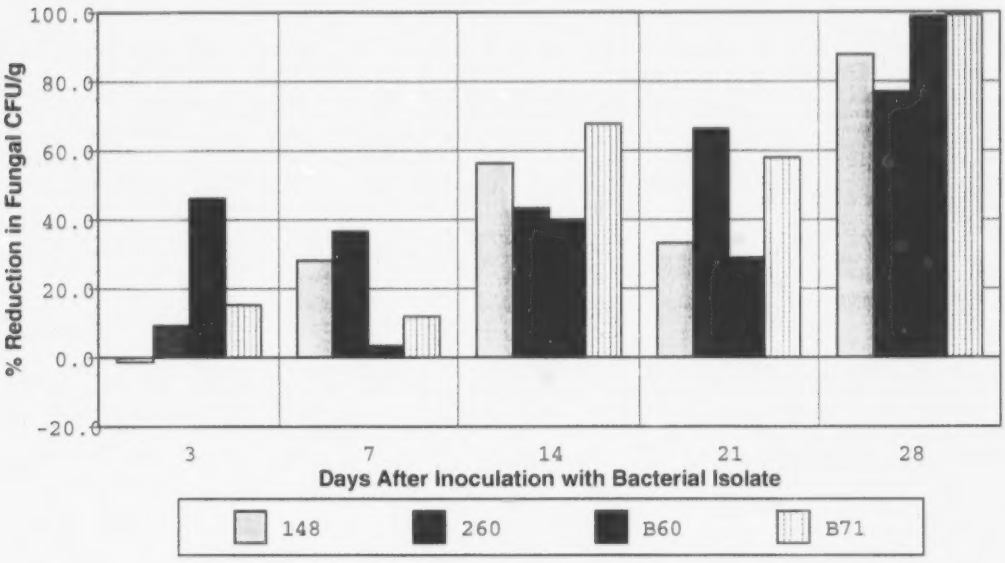

Figure 7. Suppression of *F. graminearum* CFU recovered from wheat straw by select bacterial isolates.

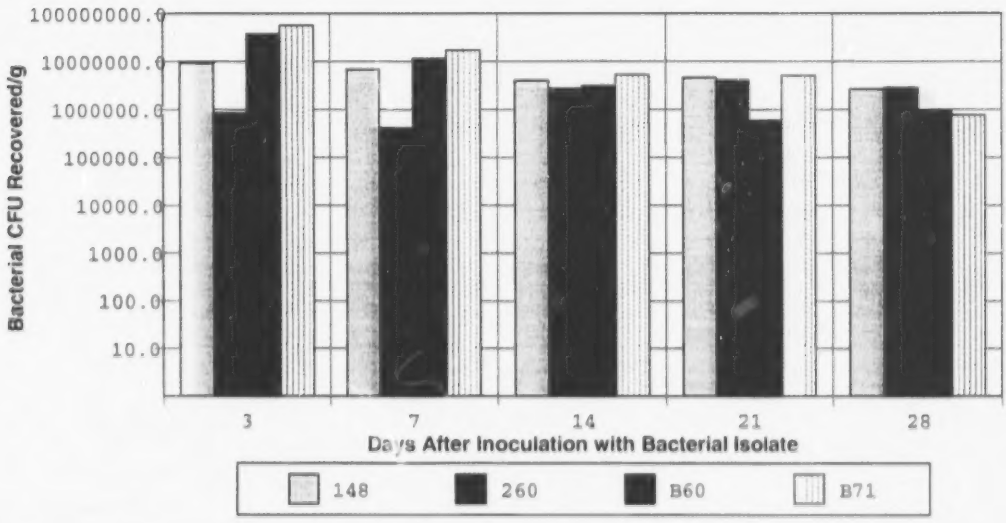

Figure 8. Bacterial populations over time on wheat straw infected with *F. graminearum*.

One of the problems associated with these assays was the risk of contamination to treatments over the course of the study. The detection of bacterial contamination was easily determined due to the distinct colonial morphology of the bacterial isolates (Plate 4). In all cases, bacterial contamination was found on one or more treatments prior to the desired completion date of 6-8 weeks. Aseptic technique was adhered to through out this process however with the multiple sub-sampling that occurred over the course of the assay the chances of contamination were more likely.

2.3 General Conclusions

Isolation of bacteria antagonistic to plant pathogenic fungi from crop materials or soil was successful. Approximately 10% of bacterial cell lines screened showed some level of suppression of vegetative fungal growth *in vitro*. Of the total number of antagonistic bacteria only 16 were found to control multiple fungal species, maintain their activity or survive storage conditions.

Evaluation of the bacterial isolates using non-sterile artificially infected wheat straw was found to be ineffective. The large number of saprophytic fungi overwhelmed the *Fusarium* spp. added and thus determining bacterial effects on the target fungi was impossible.

Using sterile wheat straw that was then inoculated with *F. graminearum* or *F. avenaceum* proved to be useful for a dual culture assay. From this evaluation, it appears that isolates B71 and 260 do not compete well with the target fungal pathogen on wheat residues. Isolate 148 tended to consistently keep its population high for about two weeks before starting to drop off. Isolate B60 showed excellent survival and control of *F. graminearum* or *F. avenaceum* on crop residues.

From this study, isolate B60 clearly emerged as the bacterial cell line with the characteristics required for a biological control agent. It was able to suppress the growth of all fungal species and isolates it was tested against. It survived at consistently high levels on wheat straw while reducing target *Fusarium* spp. significantly.

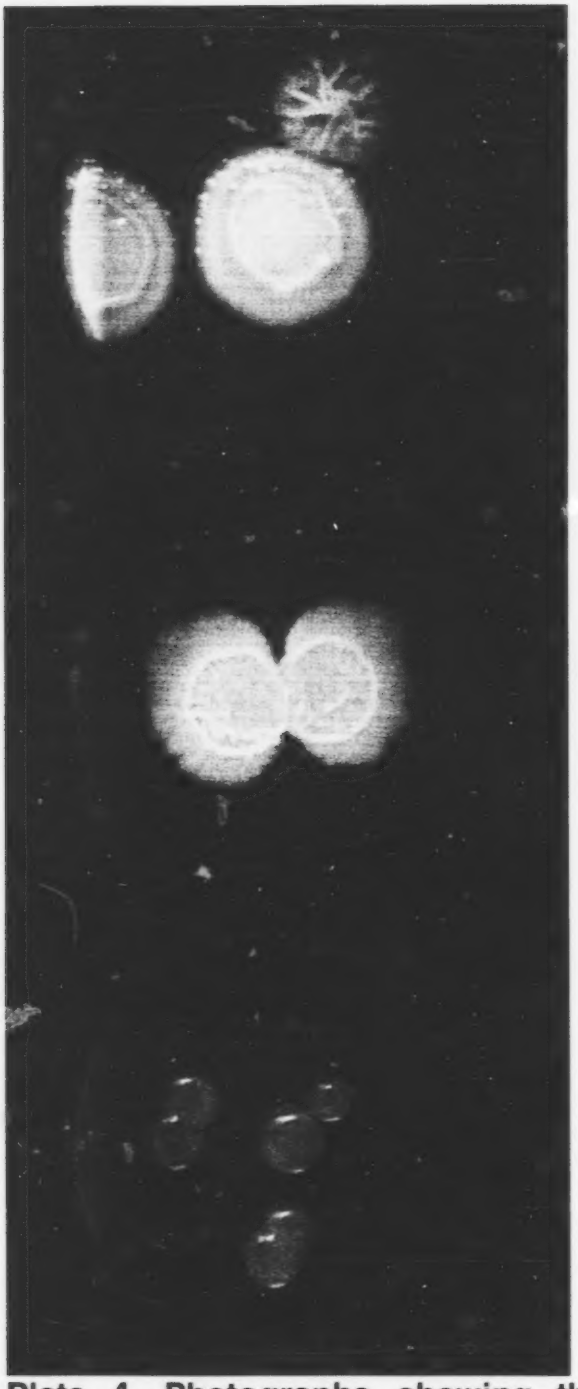

Plate 4. Photographs showing the colonial morphology of isolates 260 (top), B60 (middle) and B71 (bottom). Isolate 148 had translucent colonies that were not able to be photographed.

3. Personnel

Technical:	D. C. Wiebe J. Loehr (until December 31, 2000)
Research Team:	K. G. Hanson D. C. Wiebe Dr. M. R. Fernandez Dr. M. Banerjee (until March 30, 2001)

Project Developed Materials

A bacterial culture collection maintained in cryostorage that may be used for future work related to crop protection issues.

A poster entitled "Screening potential biocontrol microorganisms affective against *Fusarium graminearum* and related cereal pathogens" by K.G. Hanson and M.R. Fernandez was presented at the Fusarium Head Blight Forum 2001, November 2-4, 2001 in Ottawa, ON.

A poster entitled "Preliminary investigations of potential biocontrol agents effective against cereal pathogens" by K.G. Hanson and M.R. Fernandez was presented at the Saskatchewan Regional meeting of the Canadian Phytopathological Society, November 29, 2001 in Saskatoon, SK.

A poster entitled "Evaluation of bacterial strains for control of *Fusarium graminearum* and other cereal pathogens" by K.G. Hanson and M.R. Fernandez was presented at the Soils and Crops Workshop 2002, February 21 & 22, 2002 in Saskatoon, SK.

A poster entitled "Evaluation of potential biocontrol agent bacteria screened for reduction of Fusarium head blight inoculum" by K.G. Hanson and M.R. Fernandez was presented at the National meeting of the Canadian Phytopathological Society, June 16-19, 2002 in Waterton, AB.

A poster entitled "Potential biological control of Fusarium head blight fungi by antagonistic bacteria" by K.G. Hanson and M.R. Fernandez was presented at the Saskatchewan Regional meeting of the Canadian Phytopathological Society, December 4, 2002 in Saskatoon, SK.

. **Acknowledgement**

Funding by ADF was gratefully acknowledged on all presentations described above.

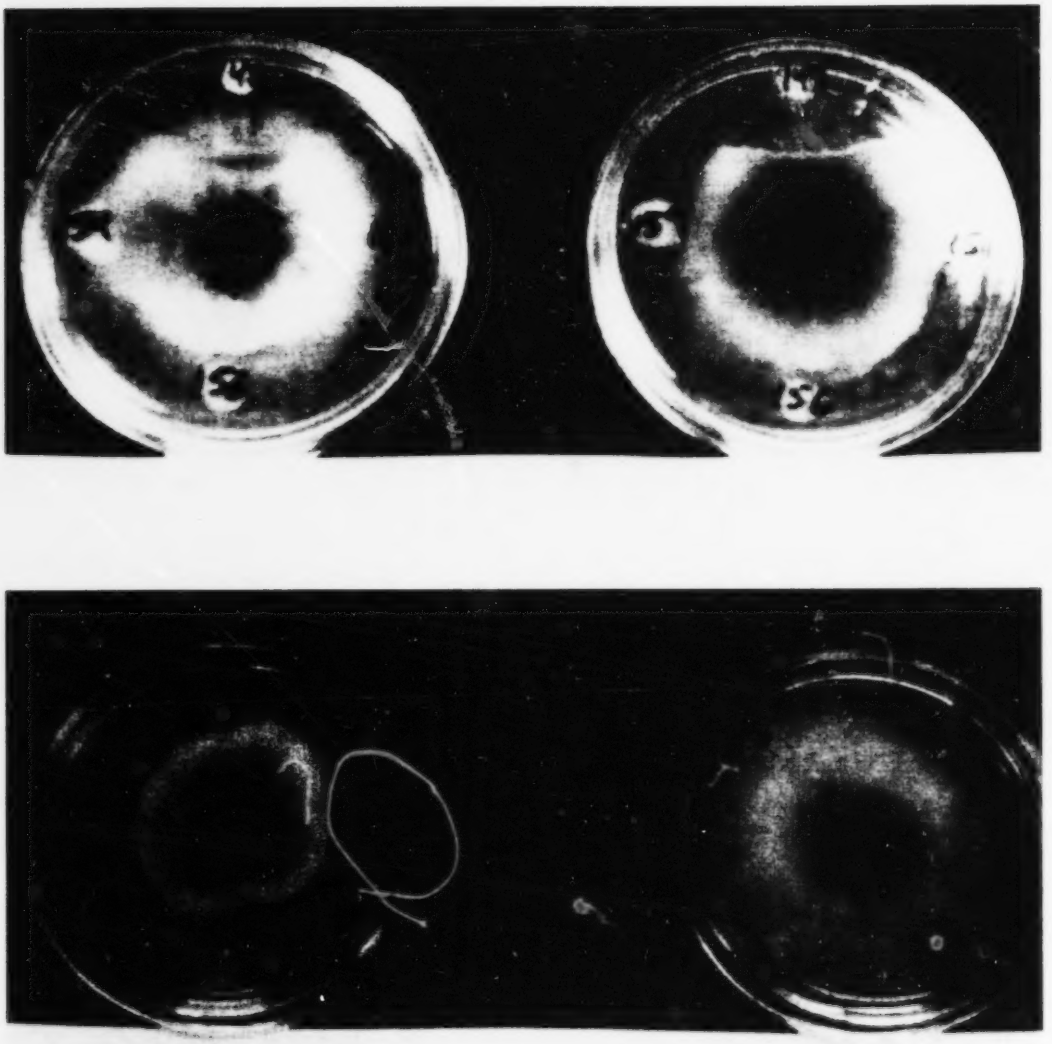

Plate 1. Photographs showing the *in vitro* screening assay. The bacterial isolates were inoculated at the periphery of the PDA plates, and the fungal isolate at the center. Bacterial isolate 148 showed good fungal antagonism (top photo). Fg I=*Fusarium pseudograminearum*, Fg II=*F. graminearum*,Fc= *F. culmorum*.

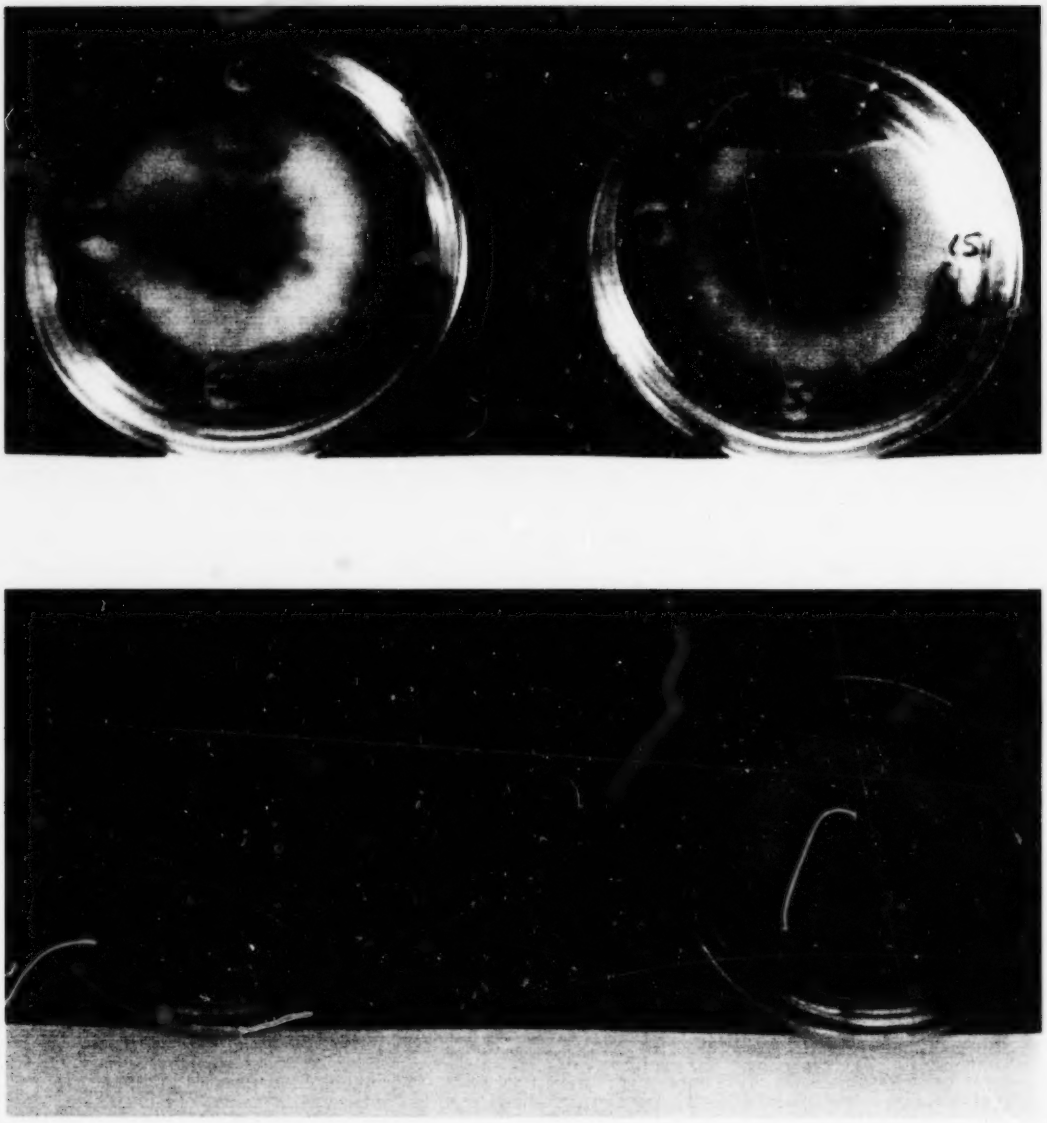

Plate 1. Photographs showing the *in vitro* screening assay. The bacterial isolates were inoculated at the periphery of the PDA plates, and the fungal isolate at the center. Bacterial isolate 148 showed good fungal antagonism (top photo). Fg I=*Fusarium pseudograminearum*, Fg II=*F. graminearum*,Fc= *F. culmorum*.

Table 1. Select bacterial isolates from initial screening and their fungal antagonism *in vitro*.

SOURCE	BACTERIAL ISOLATE	FUNGI INHIBITED (2 of each species tested)[z]					
		Fg	*Fp*	*Fc*	*Fa*	*Pt-r*	*Cs*
Wheat heads	148	2	2	2	2	2	2
Wheat heads	260	2	2	2	2	2	2
Barley rhizosphere	564	2	2	2	2	2	2
Wheat residue	B22	2	2	2	2	2	2
Wheat residue	B48	2	2	2	2	2	2
Wheat heads	B60	2	2	2	2	2	2
Wheat residue	117	2	2	2	2	1	2
Barley residue	B75	2	2	2	0	2	1
Wheat residue	B61	2	2	0	1	1	1
Wheat residue	B71	2	2	0	1	0	2
Barley residue	171	0	2	0	2	2	0
Wheat residue	177	0	2	0	2	2	0
Wheat residue	B14	1	1	2	0	2	0
Wheat residue	B17	2	2	0	0	2	0
Wheat residue	B57	1	2	0	0	0	2
Barley residue	161	0	0	0	0	2	2
Barley heads	181	0	0	0	2	2	0
Wheat rhizosphere	625	1	2	0	0	0	1
Wheat residue	159	0	0	0	1	0	2
Barley residue	160	0	0	0	1	0	2

[z] *Fg = Fusarium graminearum; Fp = F. pseudograminearum; Fc = F. culmorum; Fa = F. avenaceum; Pt-r = Pyrenophora tritici-repentis; Cs = Cochliobolus sativus*

Table 2. *In vitro* antagonism of *Fusarium graminearum* isolates from different source locations by select bacteria

BACTERIAL ISOLATE	NUMBER OF FUNGI INHIBITED		
	Manitoba Sources (10)	Alberta Sources (10)	Total (20)
148	10	10	20
260	10	10	20
B60	10	10	20
B71	10	10	20
161	10	10	20
171	10	10	20
B22	10	10	20
B57	10	10	20
B75	10	10	20
181	10	9	19
B14	9	10	19
B61	8	10	18
177	8	7	15
B17	4	9	13
564	7	5	12
117	5	6	11

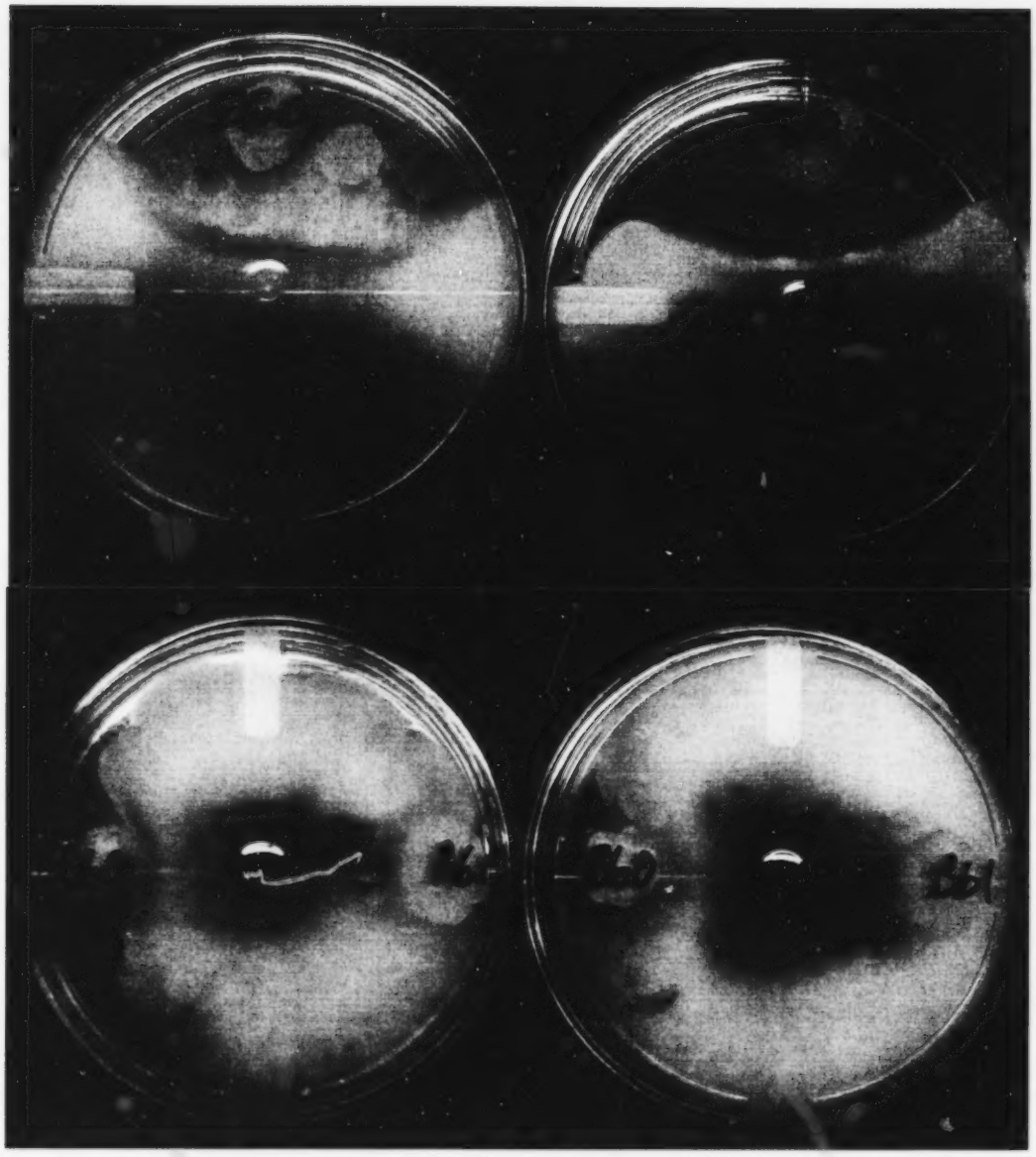

Plate 2. Photographs showing control of mycelium growth of *Fusarium graminearum* isolates from Manitoba by bacterial cultures 260 (top) and B60. Note the large zone of fungal inhibition by 260.

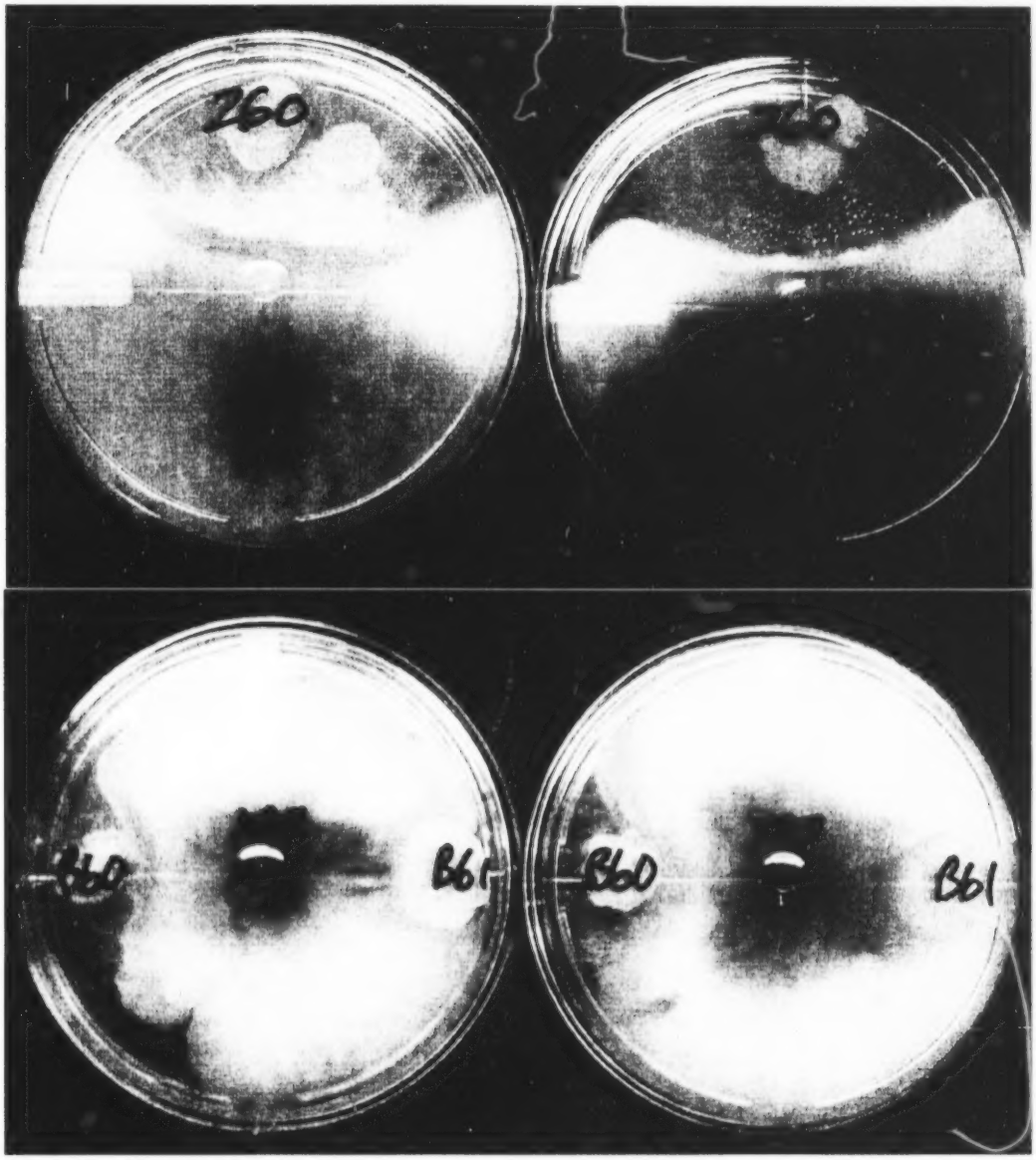

Plate 2. Photographs showing control of mycelium growth of *Fusarium graminearum* isolates from Manitoba by bacterial cultures 260 (top) and B60. Note the large zone of fungal inhibition by 260.

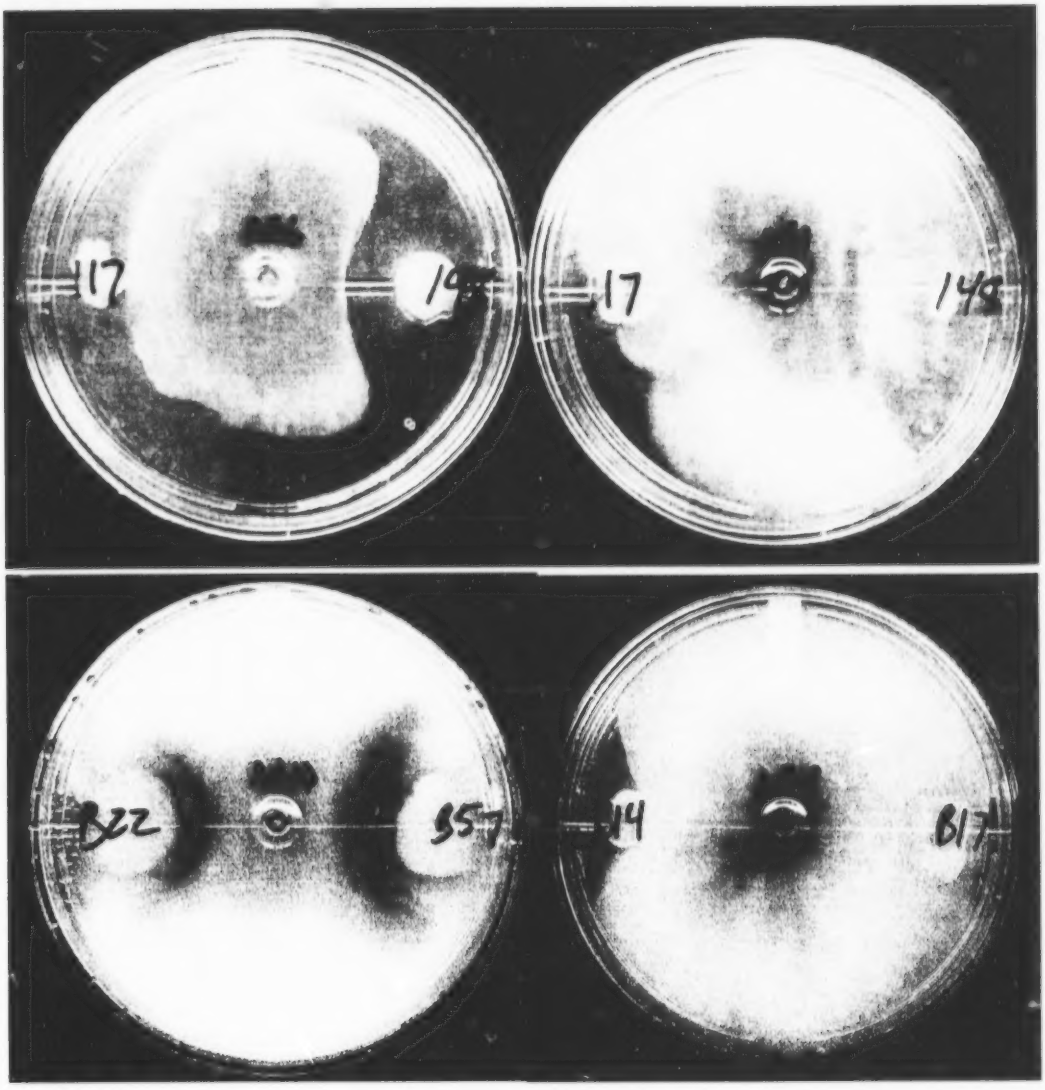

Plate 3. Photographs showing control of mycelium growth of *Fusarium graminearum* isolates from Alberta by select bacterial cultures. Note the variety of growth patterns exhibited by the fungal isolates when interacting with the different bacterial cultures.

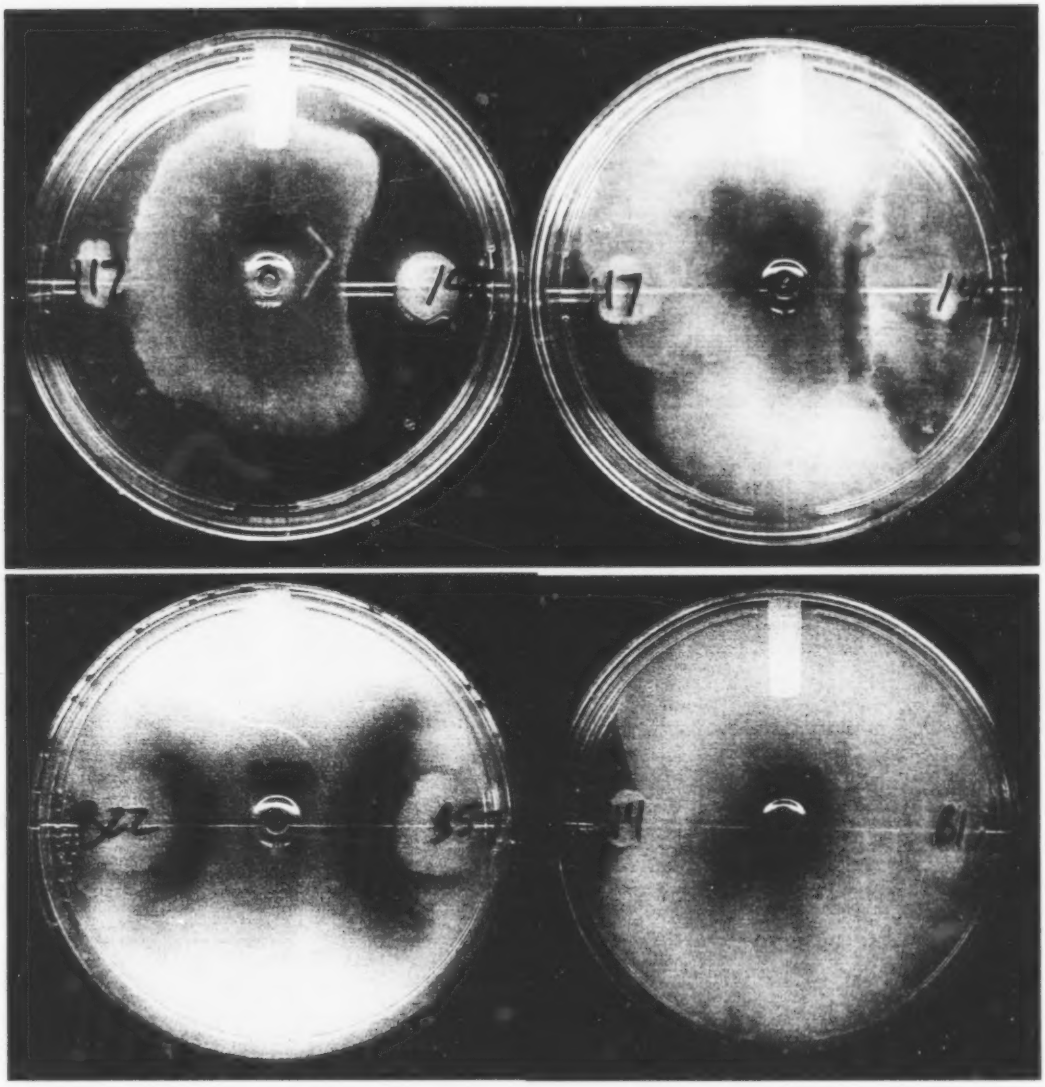

Plate 3. Photographs showing control of mycelium growth of *Fusarium graminearum* isolates from Alberta by select bacterial cultures. Note the variety of growth patterns exhibited by the fungal isolates when interacting with the different bacterial cultures.

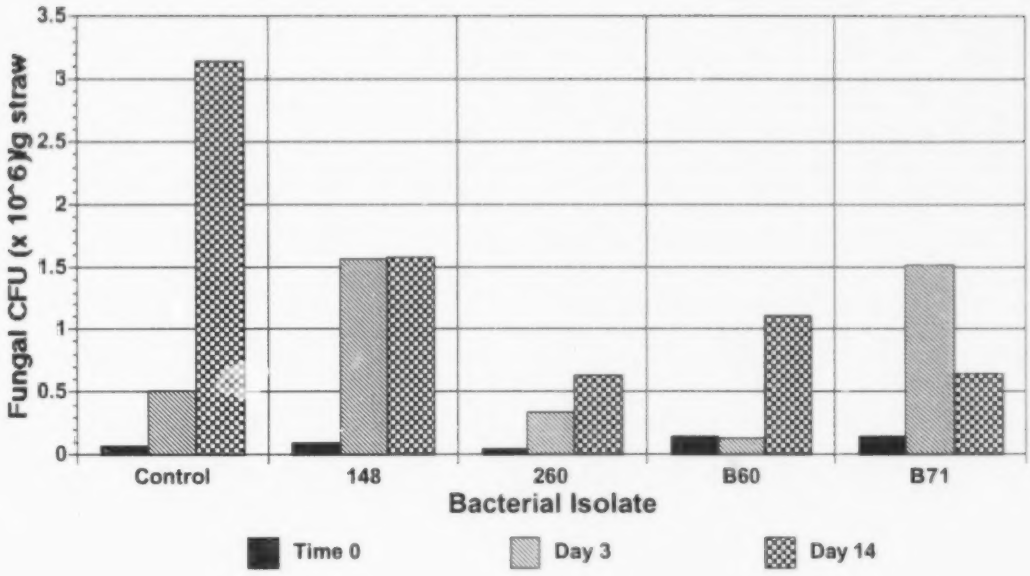

Figure 2. Recovery of total fungal colony-forming units (CFU) from *Fusarium graminearum*-infected AC Barrie residue after treatment with select bacterial isolates.

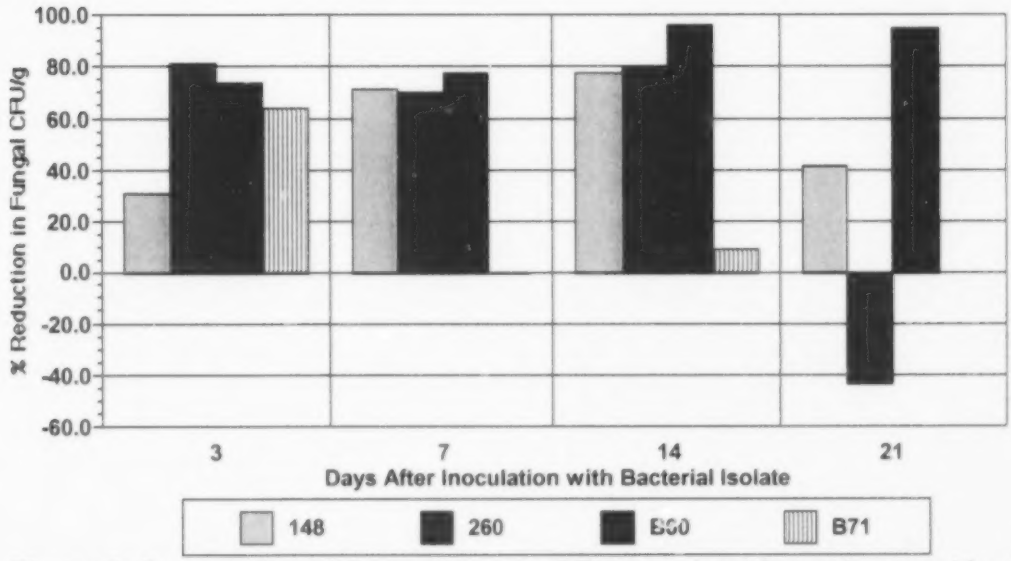

Figure 3. Suppression of *F. graminearum* CFU recovered from wheat residue by select bacterial isolates.

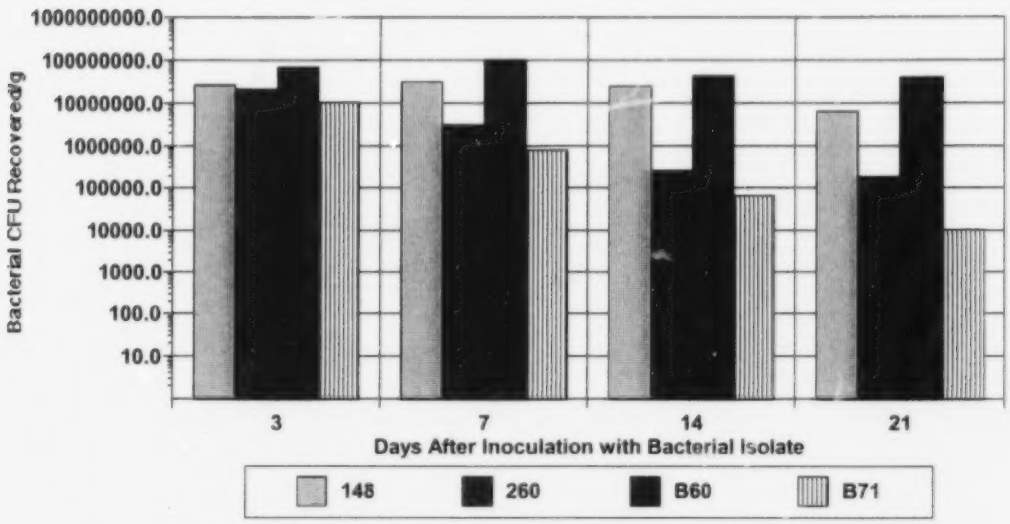

Figure 4. Bacterial populations over time on wheat residue infected with *F. graminearum*.

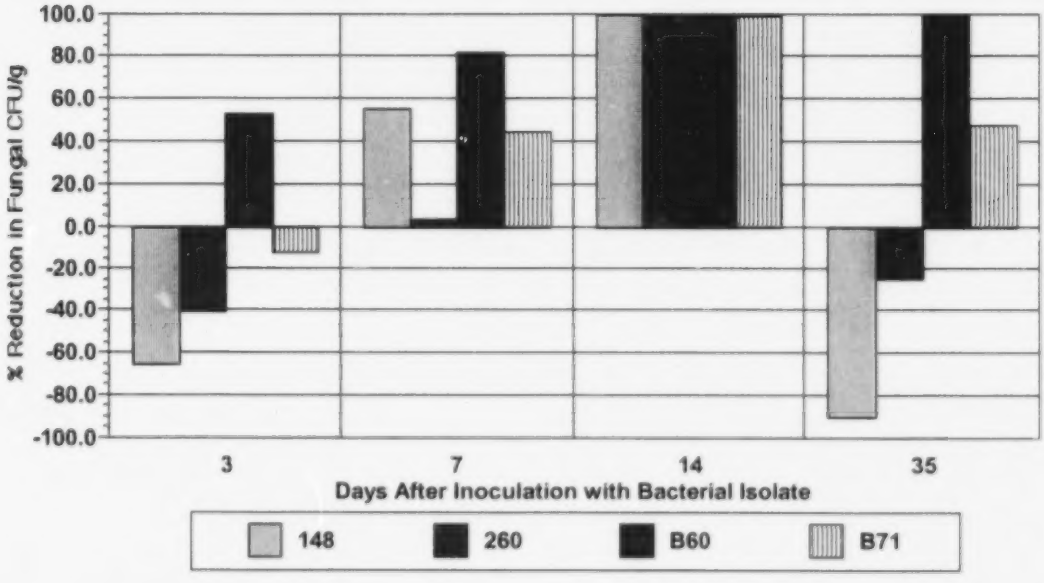

Figure 5. Suppression of *F. avenaceum* CFU recovered from wheat residue by select bacterial isolates.

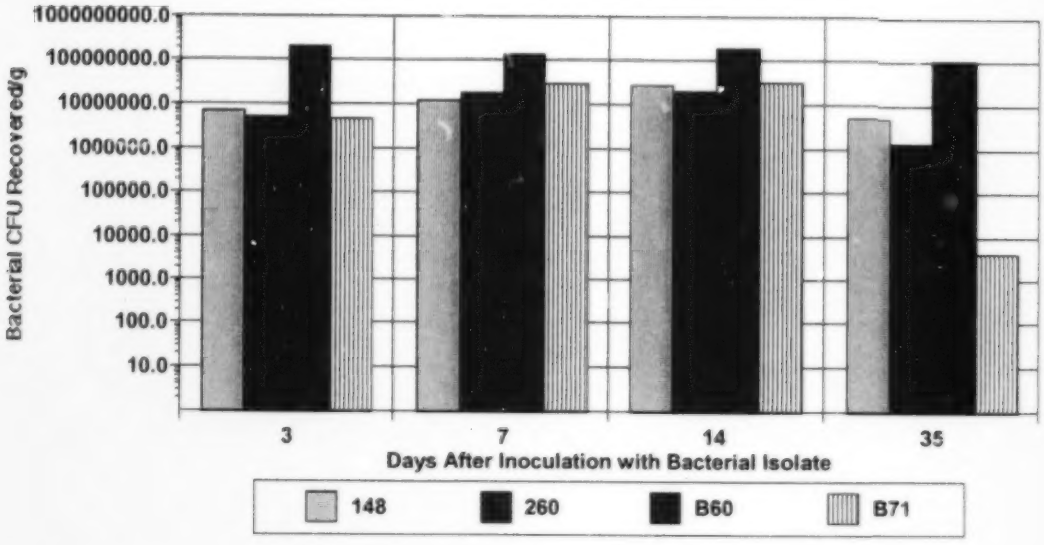

Figure 6. Bacterial populations over time on wheat residue infected with *F. avenaceum*.

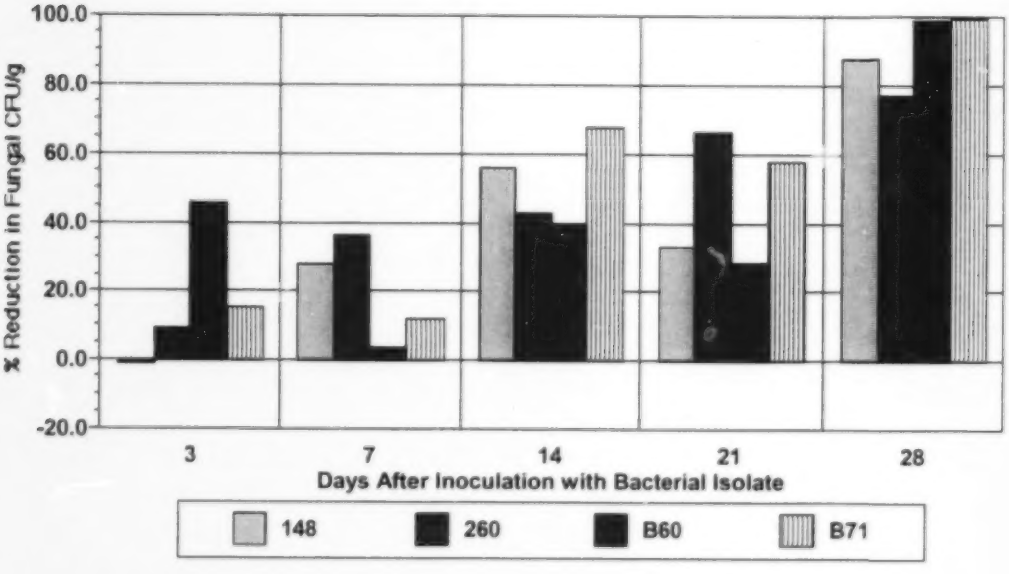

Figure 7. Suppression of *F. graminearum* CFU recovered from wheat residue by select bacterial isolates under high moisture conditions.

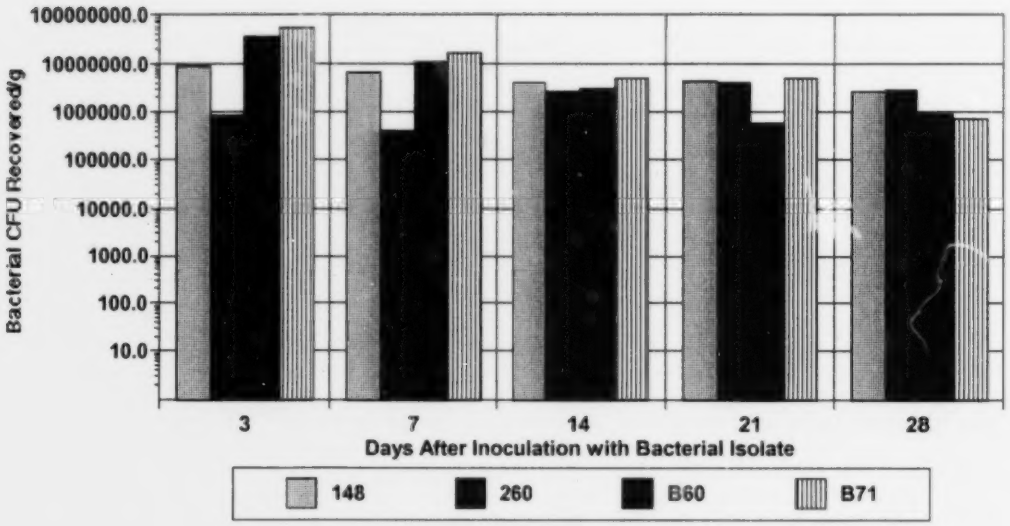

Figure 8. Bacterial populations over time on wheat residue infected with *F. graminearum* under high moisture conditions.